V. CLAIMS

What is claimed is:

1. A plasmid for use with clustered regularly interspaced short palindromic repeat (CRISPR)/ CRISPR-associated 9 (Cas9) integration systems wherein the plasmid comprises in order a left homology arm, a splice acceptor, a transgene, and a right homology arm, and further comprises a polyadenylation signal between the transgene and the right homology arm;

wherein the left and right homology arms specifically hybridize to an adeno-associated virus integrated site q (AAVS1) of chromosome 19 of humans and each has a length of 30-1000bp, and~~are each 800bp in length or less~~

wherein the right homology arm comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7, and left homology arm comprises a nucleic acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8.

~~2. The plasmid of claim 1, further comprising a polyadenylation signal between the transgene and the right homology arm.~~

~~3~~2. The plasmid of claim 1~~ or 2~~, wherein the left homology arm and right homology arm are the same length.

4~~3~~. The plasmid of claim ~~3~~2, wherein the homology arms are 30bp in length, 300bp in length, 500bp in length, or 800bp in length.

~~5~~4. The plasmid of claim 1~~ or 2~~, wherein the left homology arm and right homology arm are different lengths.

~~6. A plasmid for use in CRISPR/Cas9 integration methods consisting of 1 or 2 protospacer adjacent motifs (PAMs) and CRISPR RNAs (crRNAs) and a transgene; wherein the order of the encoded nucleic acid comprises a PAM, a crRNA and a transgene; and wherein when 2 PAMs and crRNAs are used, of the encoded nucleic acid comprises a first PAM, a first crRNA, a transgene, a second PAM, and second gRNA.~~

~~7. The plasmid of any one of claims 1-6, wherein the homology arms of the plasmid of any one of claims 1-5 and the PAMs of the plasmid of claim 6 specifically hybridize to the Adeno-Associated Virus Integration Site 1 (AAVS1) of chromosome 19 of humans.~~

~~8~~5. The plasmid of any one of claims 1-~~7~~4, wherein the transgene comprises a

polynucleotide encoding a chimeric antigen receptor for a tumor antigen.

~~9~~6. An Adeno-associated viral (AAV) vector comprising the plasmid of any one of claims 1-~~8~~5.

~~10~~7. The AAV vector of claim ~~9~~6, wherein the serotype of the AAV comprises AAV6.

~~11~~8. The AAV vector of claim ~~9~~ 6 or ~~10~~7, wherein the vector further comprises a plasmid encoding a crRNA, tracer RNA (trcrRNA), and a CAS endonuclease.

~~12~~9. The AAV vector of any of claims ~~9~~6-~~11~~8, wherein the vector is a single stranded AAV (ssAAV) and/or a self-complimentary AAV (scAAV).

~~13~~10. A modified natural killer (NK) cell comprising the plasmid of any one of claims 1-~~8~~ 5 or the AAV vector of any one of claims ~~9~~6-~~12~~9.

~~14~~11. The modified NK cell of claim ~~13~~10, wherein the modified cell is a chimeric antigen receptor (CAR) NK cell that targets a cancer antigen.

~~15~~12. Use of ~~a~~ the modified NK cell of claim ~~13~~ 10 or ~~14~~ 11 for the manufacture of a medicament for the treatment of a subject with a cancer.

~~16~~13. An ex vivo method of preparing a genetically modified natural killer (NK) ~~modifying a~~ cell by homologously directed repair comprising

a) obtaining a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease ~~(Cas9)~~ complexed with a corresponding CRISPR/Cas guide RNA and an adeno-associated virus (AAV) vector comprising a plasmid comprising a transgene ~~(such as, for example,~~that includes a chimeric antigen receptor for a tumor antigen~~)~~; wherein the transgene is flanked by homology arms~~; and wherein the homology arms~~ that are 30bp to 800bp in length ~~or less~~; and

b) introducing the transgene and the RNP complex into the NK cell; wherein the transgene is introduced into the NK cell via infection with the ~~Adeno-associated virus (~~AAV~~)~~ into the cell; wherein the RNP complex hybridizes to a target sequence within the genomic DNA of the NK cell and the NK cell's DNA repair enzymes insert the transgene into the host genome at the target sequence within the genomic DNA of the NK cell thereby creating a modified NK cell.

~~17~~14. The method of claim ~~16~~13, wherein the NK cell~~s are~~ is a primary or expanded cell~~s~~.

~~18~~15. The method of claim ~~17~~14, wherein the NK cell is a primary cell and the primary cell is~~cells are~~ incubated for 4 days in the presence of IL-2 prior to infection.

~~19~~16. The method of claim ~~18~~15, wherein the ~~cells are~~NK cell is a primary cell~~s~~ that ~~are~~ is further expanded for 4 days in the presence of irradiated feeder cells prior to infection.

~~20~~17. The method of any one of claims ~~16~~13-~~18~~15, further comprising expanding the NK cell~~s~~ with irradiated mbIL-21 expressing feeder cells, PM2l particles, EX2l exosomes, or any combination thereof following infection.

~~21. The method of any one of claims 16-20, wherein the cell is an NK cell.~~

~~22. The method of any one of claims 16-21, wherein the transgene is a chimeric antigen receptor for a tumor antigen.~~

~~23~~18. The method of any one of claims ~~16~~13-~~22~~17, wherein the RNP complex is introduced into the NK cell via electroporation, or wherein the RNP complex is introduced into the NK cell via transfection and the RNP complex is encoded on the same or a different AAV.

~~24~~19. An ex vivo method of preparing a genetically modified natural killer (NK)~~modifying at least one~~ cell by homology directed repair (HDR) comprising:

(a) introducing into the NK cell (i) a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease complexed with a CRISPR/Cas guide RNA encoding a target sequence in the cell genome, and (ii) an AAV vector comprising a plasmid comprising a transgene flanked by first and second homology arms each having a length of no more than 800bp, wherein the transgene encodes a chimeric antigen receptor; and

(b) maintaining the NK cell for a time and under conditions sufficient for (i) the RNP complex to hybridize to the target sequence and introduce a double-stranded break (DSB) in the target sequence; and (ii) the NK cell to insert the transgene into ~~the cell~~its genome at the target sequence and repair the break by HDR, thereby incorporating the transgene into the NK cell genome at the site of the DSB.

~~25~~20. The method of claim ~~24~~19, wherein the NK cell is a primary or an expanded cell.

~~26. The method of claim 24 or 25, wherein the cell is a natural killer (NK) cell.~~

~~27~~21. The method of claim ~~24~~ 19 or ~~25~~20, wherein the class 2 CRISPR/Cas endonuclease is Cas9.

~~28~~22. The method of claim ~~24~~ 19 or ~~25~~20, wherein the NK cell is a primary cell and the method further comprises incubating the NK cell at least ~~about~~ 4 days in the presence of membrane bound IL-21 (mbIL-21) prior to step (a).

~~29~~23. The method of claim ~~24~~ 19 or ~~25~~20, wherein the NK cell is a primary cell and the method further comprises expanding the NK cell for at least ~~about~~ 4 days in the presence of mbIL-21 after step (b).

~~30~~24. The method of claim ~~29~~23, wherein the mbIL-21 comprises irradiated mbIL-21 expressing feeder cells, PM21 particles, EX21 exosomes, or any combination thereof.

25. A plasmid for use with clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) integration systems, wherein the plasmid comprises a sequence selected from SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.